

1 WHAT IS CLAIMED IS:  
2

- 3 1. A method for detection of at least one allele of a  
4 genetic locus comprising amplifying genomic DNA  
5 with an intron-spanning primer pair that defines a  
6 DNA sequence, said DNA sequence being in genetic  
7 linkage with said genetic locus and containing a  
8 sufficient number of intron sequence nucleotides  
9 to produce an amplified DNA sequence  
10 characteristic of said allele.  
11 2. The method of Claim 1 wherein said amplified DNA  
12 sequence includes at least about 300 nucleotides  
13 corresponding to intron sequences.  
14 3. The method of Claim 1 wherein said intron sequence  
15 is adjacent to an exon encoding said allele.  
16 4. The method of Claim 1 wherein said amplified DNA  
17 sequence is characteristic of at least one  
18 nonadjacent allele.  
19 5. The method of Claim 1 wherein said amplified DNA  
20 sequence is characteristic of at least one  
21 adjacent allele and at least one nonadjacent  
22 allele.  
23 6. The method of Claim 5 wherein said amplified DNA  
24 sequence includes at least about 1,000 nucleotides  
25 corresponding to intron sequences.  
26 7. A method for detection of at least one allele of a  
27 genetic locus comprising:  
28 a. amplifying genomic DNA with an intron-  
29 spanning primer pair that defines a DNA  
30 sequence, said DNA sequence being in genetic  
31 linkage with said allele and containing a  
32 sufficient number of intron sequence  
33 nucleotides to produce an amplified DNA  
34 sequence characteristic of said allele; and

1 b. analyzing said amplified DNA sequence to  
2 detect the presence of a genetic variation in  
3 said amplified sequence.

4 <sup>10</sup>8. The method of Claim 7 wherein said variation in  
5 said amplified DNA sequence is a variation in the  
6 length of the primer-defined amplified DNA  
7 sequence.

8 <sup>11</sup>9. The method of Claim 7 wherein said variation in  
9 said amplified DNA sequence is a change in the  
10 presence of at least one restriction site in the  
11 primer-defined amplified DNA sequence.

12 <sup>12</sup>10. The method of Claim 7 wherein said variation in  
13 said amplified DNA sequence is a change in the  
14 location of at least one restriction site in the  
15 primer-defined amplified DNA sequence.

16 <sup>13</sup>11. The method of Claim 7 wherein said variation in  
17 said amplified DNA sequence is a substitution of  
18 at least one nucleotide in the primer-defined  
19 amplified DNA sequence.

20 <sup>14</sup>12. The method of Claim 7 wherein said genetic locus  
21 is a major histocompatibility locus.

22 <sup>15</sup>13. The method of Claim 7 wherein said allele is  
23 associated with a monogenic disease.

24 <sup>16</sup>14. The method of Claim 13 wherein said monogenic  
25 disease is cystic fibrosis.

26 <sup>17</sup>15. The method of Claim 7 wherein at least about 70%  
27 of said primer-defined amplified DNA sequence  
28 corresponds to intron sequences.

29 <sup>18</sup>16. The method of Claim 7 wherein said primer-defined  
30 amplified DNA sequence is from 300 to 500  
31 nucleotides in length.

32 17. A method for producing RFLP fragments for an HLA  
33 locus of an individual comprising the steps of:

34 a. amplifying genomic HLA DNA from said  
35 individual with a primer pair specific for

1 said HLA locus under conditions suitable to  
2 produce an amplified DNA sequence; and  
3 b. producing a digest by combining said  
4 amplified DNA sequence with at least one  
5 endonuclease that cleaves said amplified DNA  
6 sequence to yield a set of fragments having  
7 distinctive fragment lengths.

8 18. The method of Claim 17 additionally comprising the  
9 step of producing RFLP patterns from said digest.

10 19. The method of Claim 17 wherein said primers define  
11 a DNA sequence that contains all exons that encode  
12 allelic variability associated with said HLA  
13 locus.

14 20. A method for producing RFLP fragments for an HLA  
15 locus of an individual comprising the steps of:

16 a) amplifying genomic HLA DNA from said  
17 individual with a primer pair specific for  
18 said HLA locus under conditions suitable to  
19 produce an amplified DNA sequence, said  
20 primers defining a DNA sequence that contains  
21 all exons that encode allelic variability  
22 associated with said HLA locus; and  
23 b) producing a digest by combining said  
24 amplified DNA sequence with at least one  
25 endonuclease that cleaves said amplified DNA  
26 sequence to yield a set of fragments having  
27 distinctive fragment lengths.

28 21. A method for producing RFLP patterns for an HLA  
29 locus of an individual comprising the steps of:

30 a) amplifying HLA DNA from said individual with  
31 a primer pair specific for said HLA locus  
32 under conditions suitable to produce an  
33 amplified DNA sequence, said primers being  
34 located in intervening sequence I and in  
35 intervening sequence III when said HLA locus  
36 is a Class I locus and in intervening

1 sequence I and in intervening sequence II  
2 when said locus is a Class II locus;  
3 producing a digest by combining said  
4 amplified DNA sequence with at least one  
5 endonuclease that cleaves said amplified DNA  
6 sequence to yield a set of fragments having  
7 distinctive fragment lengths; and  
8 e. producing RFLP patterns from said digest.

9 22. The method of Claim 21 wherein said amplification  
10 comprises:

- 11 a. combining an HLA-locus specific primer pair  
12 with HLA DNA from said individual under  
13 hybridizing conditions for a period of time  
14 sufficient for each primer in said primer  
15 pair to produce an extension product which,  
16 when separated from its complement, can serve  
17 as a template for synthesis of the extension  
18 product of the other primer to produce a  
19 mixture;  
20 b. treating said mixture under denaturing  
21 conditions to separate the primers from their  
22 extension products;  
23 c. treating said mixture with said HLA locus-  
24 specific primer pair such that a primer  
25 extension product is synthesized using each  
26 of the templates produced in step (b) as a  
27 template, resulting in amplification of the  
28 HLA DNA; and  
29 d. repeating steps (b) and (c) to produce an  
30 amplified DNA sequence.

31 23. The method of Claim 21 wherein a second primer  
32 pair specific for said HLA locus is also used to

33 amplify said HLA DNA,

34 24. The method of Claim 21 wherein producing said RFLP  
35 fragment pattern comprises:

- c 1        i) combining said amplified DNA sequence with at  
2        a least one endonuclease that cleaves said  
3        amplified DNA sequence to yield a set of  
4        fragments having distinctive fragment  
5        lengths;  
c 6        ii) separating said fragments based on the length  
7        a of the fragments to produce separated  
8        fragments; and  
c 9        iii) visualizing said separated fragments to  
10        a produce RFLP fragment patterns.

11        25. <sup>23</sup> The method of Claim <sup>22</sup> 24 wherein said fragments are  
12        separated using gel electrophoresis and visualized  
13        using a nucleotide-specific stain.

14        26. A method for determining whether DNA in a sample  
15        is from a particular individual comprising the  
16        steps of:

c 17        a) amplifying DNA from said individual and DNA  
18        a from said sample with a primer pair specific  
19        for an HLA locus under suitable conditions to  
20        produce an amplified DNA sequence from said  
21        individual and from said sample, said primers  
22        being located in intervening sequences I and  
23        III for an HLA Class I locus and in  
24        intervening sequences I and II for a Class II  
25        locus;

c 26        b) combining said amplified DNA sequence from  
27        a said individual and said amplified sample DNA  
28        from said sample with at least one  
29        endonuclease that cleaves said amplified DNA  
30        sequence into a plurality of cleaved  
31        sequences of sufficiently different lengths  
32        to distinguish between alleles of said HLA  
33        locus for a period of time sufficient for  
34        digestion of said amplified DNA to produce a  
35        digest; and

c 1 Sub E4 7 c) comparing restriction fragment length  
2 polymorphic patterns produced by said digest  
3 from said individual and from said sample.

4 27. A method for determining whether an individual is  
5 the father of a child comprising the steps of:

- 6 a. amplifying DNA from said individual, DNA from  
7 said child and DNA from said child's mother  
8 with a pair of primers specific for an HLA  
9 locus under suitable conditions to produce  
10 amplified DNA sequences, said primers being  
11 located in intervening sequences I and III  
12 for an HLA Class I locus and in intervening  
13 sequences I and II for a Class II locus;  
14 b. combining said amplified DNA sequence from  
15 said individual and said amplified sample DNA  
16 from said child with at least one  
17 endonuclease that cleaves said amplified DNA  
18 sequence into a plurality of cleaved  
19 sequences of sufficiently different lengths  
20 to distinguish between alleles of said HLA  
21 locus to produce a digest; and  
22 c. comparing restriction fragment length  
23 polymorphic patterns produced by said digest  
24 from said individual, from said child's  
25 mother and from said child.

26 28. An HLA locus-specific primer selected from the  
27 group consisting of a Class I locus-specific  
28 primer, a Class I A locus-specific primer, a Class  
29 I B locus-specific primer and a Class I C locus-  
30 specific primer.

31 29. The HLA locus-specific primer of Claim 28 wherein  
32 said primer has a sequence corresponding to at  
33 least 15 consecutive nucleotides selected from the  
34 group consisting of CATGTGGCCATCTTGAGAATGGA;  
35 GCCCGGGAGATCTACAGGCGATCA; CGCCTCCCTGATCGCCTGTAG;  
36 CCAGAGAGTGACTCTGAGG; CACAATTAAGGGAT;

1 TCCCCGGCGACCTATAGGAGATGG; CTAGGACCACCCATGTGACCAGG;  
 2 ATCTCCTCAGACGCCGAGATGCGTCAC;  
 3 CTCCTGCTGCTCTGGGGGGCAG; ACTTTACCTCCACTCAGATCAGGAG;  
 4 CGTCCAGGCTGGTGTCTGGGTTCTGTGCCCCCT;  
 5 CTGGTCACATGGGTGGTCCTAGG;  
 6 CGCCTGAATTTTCTGACTCTTCCCAT;  
 7 ATCCCCGGGAGATCTACAGGAGATG; AACAGCGCCCATGTGACCATCCT;  
 8 CTGGGGAGGCGCCGCGTTGAGGATTCT;  
 9 CGTCTCCGCAGTCCCGGTTCTAAAGTTCCAGT;  
 10 ATCCTCGTGCTCTCGGGA; TGTGGTCAGGCTGCTGAC;  
 11 AAGGTTTGATTCCAGCTT;  
 12 CCCCTTCCCCACCCAGGTGTTCCCTGTCCATTCTTCAGGA;  
 13 CACATGGGCGCTGTTGGAGTGTCG; GTGAGTGCGGGGTCGGGAGGGA;  
 14 CACCCACCGGGACTCAGA; TGGCCCTGACCCAGACCTGGGC;  
 15 GAGGGTCGGGCGGGTCTCAGC; CTCTCAGGCCTTGTTT;  
 16 CAGAAGTCGCTGTTCC; TTCTGAGCCAGTCCTGAGA;  
 17 TTGCCCTGACCACCGTGATG; CTCCTGCTTGTCATCTTCA;  
 18 CCATGAATTTGATGGAGA; ACCGCTGCTACCAATGGTA;  
 19 CCAAGAGGTCCCCAGATC; TCATCATAGCTGTGCTGATG;  
 20 AGAACATGTGATCATCCAGGC; CCAACTATACTCCGATCACCAAT;  
 21 TGACAGTGACACTGATGGTGCTG; GGGGACACCCGACCACGTTTC;  
 22 TGCAGACACAACACTACGGGGTTG; TGGCTGAGGGCAGAGACTCTCCC;  
 23 TGCTACTTCACCAACGGGAC; GGTGTGCACACACAACACTAC;  
 24 AGGTATTTTACCCAGGGACCAAGAGAT;  
 25 ATGTAAAATCAGCCCGACTGCCTCTTC;  
 26 GCCTCGTGCCCTTATGCGTTTGCCTCCT;  
 27 TGAGGTTAATAAACTGGAGAA; GAGAGTGGCGCCTCCGCTCAT; and  
 28 GAGTGAGGGCTTTGGGCCGG.

- 29 30. An HLA Class I locus-specific primer pair.
- 30 31. An HLA Class II locus-specific, intron-spanning
- 31 primer pair.
- 32 32. A DNA sequence defined by an HLA locus-specific
- 33 primer pair.
- 34 33. A kit comprising at least one HLA locus-specific
- 35 primer pair in a suitable container, wherein said
- 36 HLA locus-specific primer pair is selected from

1 the group consisting of an HLA Class I locus-  
2 specific primer pair and an HLA Class II locus-  
3 specific, intron-spanning primer pair.  
4 34. The kit of Claim 33 additionally comprising at  
5 least one endonuclease that cleaves a DNA sequence  
6 defined by said HLA locus-specific primer pair  
7 into a plurality of cleaved sequences of  
8 sufficiently different lengths to distinguish  
9 between alleles of said HLA locus.  
10

add  
A5

add  
E6